



Utilizing water level draw-down to remove excess organic matter in a constructed treatment wetland

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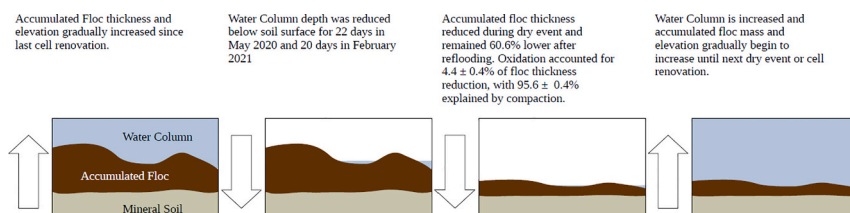
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HIGHLIGHTS

- Constructed treatment wetlands naturally accumulate organic matter.
- Organic matter removal via temporary water level draw-down was tested.
- Water level draw-down permanently reduced floc by 60 % and soil elevation by 2.7 cm.
- Most (~96 %) organic matter loss was due to consolidation.
- Incorporating water level draw-down can benefit wetland management.

GRAPHICAL ABSTRACT



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ABSTRACT

Constructed treatment wetlands are commonly used to enhance surface water nutrient removal following traditional wastewater treatment. However, the constant inflow may necessitate continuous wetland inundation, leading to persistent anaerobic conditions and the accumulation of organic matter (OM) as suspended detrital flocculent (floc) and soil OM. This study investigated if temporary water level draw-down (WLDD) could promote OM consolidation and oxidation without impacting nutrient removal efficiency. A large-scale, 2-y, before-after-control-impact field experiment at the Orlando Easterly Wetland (Christmas, FL, USA) was complemented by an intact soil core laboratory experiment with varied WLDD regimes. Changes in floc thickness, soil elevation, and surface water and soil nutrients were quantified. Field experiment results demonstrated an average floc thickness reduction of 60 % and soil elevation decline of 2.7 cm persisted after return to normal flow operation. This reduction was achieved with one ~3-week dry event for two consecutive years and removed an estimated 7.5 years' worth of accumulated floc. Intact soil core results showed a direct relationship ($R^2 = 0.93$) between days of WLDD and cumulative $\text{CO}_2\text{-C}$ loss, despite oxidation only accounting for 4–5 % of OM loss (and consolidation accounting for the remaining 95–96 %). While soil nitrogen (N) and phosphorus (P) concentrations did tend to increase during WLDD, outflow surface water N was not affected by the WLDD. Soluble reactive P increased for ~36 days following reflooding, then returned to baseline. Incorporating WLDD into wetland management every few years could significantly reduce the frequency of costly cell renovation projects aimed at removing accumulated OM.

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1. Introduction

Constructed treatment wetlands (CTWs) have been utilized worldwide for decades as an economical and effective method for removing diverse pollutants (DuPoldt et al., 1996; Kadlec and Knight, 1996). One common application of CTWs is to enhance the removal of nitrogen (N) and phosphorus (P) from treated wastewater, two ecologically important nutrients that can be difficult to fully remediate with conventional wastewater treatment (Kadlec and Knight, 1996). As naturally limited nutrients in many ecosystems, anthropogenic N and P are increasingly causing eutrophication globally, which fuel algal blooms and hypoxia within waterbodies (Anderson et al., 2002; Diaz and Rosenberg, 2008). Although these two nutrients are often targeted for remediation in tandem, their biogeochemical cycles and mechanisms for removal are very different. Nitrogen is a dynamic element that can exist in numerous valance states and undergo complex cycling in the natural environment, including multiple organic and inorganic forms, and both particulate and dissolved. In wastewater, N is commonly found as dissolved inorganic N (DIN), which includes nitrate (NO_3^-) and ammonium (NH_4^+). Although many pathways exist for N removal from the water column (e.g., ammonia volatilization, assimilation, adsorption, burial, and anaerobic ammonium oxidation), bacterial denitrification (or coupled nitrification-denitrification) is typically the predominate removal pathway in CTWs (Lund et al., 1999; Vymazal, 2007). The denitrification pathway for N removal is often targeted because the dominant end-product is N_2 , a ubiquitous, chemically inert gas that can naturally exit the system through diffusion.

In contrast to N, P lacks a common gaseous phase. Removal of P in CTWs focuses on sorption to minerals, biomass assimilation, and burial, with the dominate fraction of P being stored in the soil and sediment (Kadlec and Knight, 1996). Long-term performance of large CTWs shows a median P concentration reduction of ~71 %, but systems also tend to exhibit stochastic variability over time (Kadlec, 2016). In general, more acidic soils promote P-fixation with iron and aluminum, while alkaline soils are dominated by P sorption to calcium and magnesium; changes in pH and redox can impact the solubility and re-release of P to varying degrees based on the form (Reddy and D'Angelo, 1994).

All wetlands, including CTWs, are naturally prone to accumulate organic matter (OM) over time due to the combination of high primary production and slow decomposition caused by the anaerobic environment created by water-logged conditions (Reddy and DeLaune, 2008). This OM can include live and dead plant biomass, unconsolidated suspended flocculent or detrital material (henceforth referred to as "floc"), and soil OM that accretes vertically. Compared to natural wetlands that may flood and drain seasonally, or even daily, the hydroperiod of managed wetlands is often less variable (Mitsch and Gosselink, 2015). For example, the inflow rate may be roughly constant due to a consistent supply of wastewater in need of treatment. Likewise, the outflow rate and water level may be controlled with weirs, levees, and standpipes to optimize the loading rate and hydraulic retention time. Since periods of low or subsurface water levels are known to rapidly accelerate soil mineralization though improved oxygen diffusion, resulting in the natural oxidation of some of the accumulated OM (Chambers et al., 2013), the artificial maintenance of a near-constant water level is expected to promote OM accumulation and is maintained as the typical operational hydraulic regime for most CTWs.

Accumulated OM is important to the biogeochemical functioning of a CTW because it contains much of the sequestered P and provides ideal conditions to support denitrification (Burford and Bremner, 1975). However, when OM accumulation becomes too extreme it can cause hydrologic inefficiencies by obstructing flow, leading to preferential flow paths, the development of dead zones, and reduced water mixing (Kadlec and Knight, 1996). Optimizing the time period that water can interact with soil and accumulated organic matter is crucial to the proper functioning of CTWs due to the direct correlation between water residence time, biogeochemical nutrient cycling, and pollutant removal

(Martinez and Wise, 2003; Persson and Wittgren, 2003).

In response to excessive OM accumulation in CTWs, a variety of management activities have been implemented to remove the OM and "rejuvenate" CTWs; the ultimate goal of these activities is improved hydraulic efficiency, which will in-turn also improve nutrient removal efficiency. Management practices range from highly disruptive, such as draining entire basins or treatment cells and mechanically scooping-out all the vegetation, floc, and surface soil with heavy machinery (effectively re-starting secondary succession), to preforming prescribed burns to remove live and dead vegetation (Wang et al., 2006; White et al., 2008). While full-cell renovation methods (i.e., mechanical OM removal, re-grading, and re-vegetating; sometimes referred to as "de-mucking") have been proven to significantly improve both P retention and hydraulic efficiency in a large CTW in Orlando, FL (the Orlando Easterly Wetlands (OEW)), the economic and ecological costs can be considerable given CTWs often serve ancillary services in addition to wastewater remediation, such as wildlife habitat, aesthetics, recreation, and education (M. Sees, personal communication).

The goal of this study was to determine if temporary water-level draw-down (WLDD) is an effective management technique to reduce the volume of accumulated OM in the OEW, a municipal wastewater CTW. This goal was achieved through two complementary studies, 1) a before-after-controlled-impact (BACI) field experiment, and 2) a laboratory intact soil core experiment. The objective of the field experiment was to document the impact of WLDD on water, soil, and floc properties, as compared to a hydrologic control/reference cell. It was hypothesized that 1a) WLDD would decrease floc thickness and soil elevation relative to the control and initial measurements, 1b) these reductions would be maintained following post-experiment re-flooding, and 1c) a temporary increase in the concentration of soil extractable and surface water inorganic N and P would return to baseline conditions within 46 days post manipulation.

The objective of the laboratory core experiment was to determine the optimal design of the WLDD event to maximize soil consolidation and oxidation. Specifically, a continuously flooded Control condition was chosen, along with four treatment conditions to compare the length of the WLDD (Single Short v. Single Long), the role of wet-dry cycles (Multiple Short), and impact of adding a clay amendment during a Single Long WLDD. The Single Long Amended treatment was included for two reasons, 1) mineral-associations have been shown to afford physical and chemical protection to soil OM by preventing microbial access to C substrates (e.g., Cotrufo and Lavalley, 2022; Lavalley et al., 2018; von Lutzow et al., 2006) and organic-rich wetland soils (like those found in this CTW) are known to be limited in their ability to form mineral associated OM (MAOM) due to the low availability of fine mineral sediments (Mirabito and Chambers, 2023). 2) OEW managers have previously employed 'hybrid de-mucking' management practices where significant quantities of accumulated OM are mechanically removed, while the remaining OM was mechanically mixed (or turned over) into the underlying clay-rich mineral soil. The Single Long Amended treatment sought to evaluate the effectiveness of this 'hybrid de-mucking' approach, as compared to traditional rejuvenation methods, in light of MAOM formation. For the laboratory experiment, it was hypothesized that 2a) multiple cycles of WLDD and re-flooding would accelerate mineralization (CO_2 loss) more than a single WLDD event, 2b) floc would partially resuspend following reflooding, and 2c) the addition of a clay amendment along with WLDD will suppress CO_2 loss, when compared to the same conditions without the amendment.

2. Methods

2.1. Study location

The OEW is a 500 ha CTW managed by the City of Orlando and located in Christmas, Florida (28°34'23"N, 80°59'54"W). The OEW, first operational in 1987 and consisting of 17 wetland treatment cells ranging

in size of 6.1 ha to 52.6 ha, provides additional nutrient removal from tertiary treated wastewater effluent from the Iron Bridge Regional Water Reclamation Facility to achieve regulatory limits for total N and P prior to discharge into the St. Johns River watershed (Slayton, 2021). The wastewater inflow (up to 35 million gallons per day (MGD) of reclaimed water) is transported from Iron Bridge via underground pipe 27.4 km to the OEW, where it enters a three-way splitter box that evenly divides the water volume among three flow-trains (north, central, and south). Water then flows slowly via gravity through a series of cells designed to support four primary communities: cattail-dominated (*Typha* spp.) deep marsh, submergent and emergent mixed marsh, hardwood swamp, and a 36 ha lake. Each cell is separated by earthen berms and weirs that utilize a modest topographic gradient (~0.2 % slope) to create an average water retention time of 30–45 days before discharged through a single outflow into the St. John's River.

2.2. Field experiment design and sample collection

To achieve the goals of objective 1, two treatment cells (cell 11 and 12; Fig. 1) at the OEW were identified due to similarities in dominant vegetation, time since last renovation, time since last prescribed fire, total cell acreage, and location within the same flow-train. Specifically, both cells were dominated by *Typha* sp. and *Pistia* sp., approximately 12.1 ha in size, renovated within 2 y of 2010, burned within 2 y of 2014, and located in the first stratum of the southern flow-train. A partial earthen berm exists mid-way within of each cell and only the eastern halves of both cells were used. Fourteen sampling points were randomly chosen within each cell (28 total) using ESRI ArcGIS Pro random point tool after applying a 10 m edge buffer around each cell to limit edge-effects. Each sampling point was reached by foot or airboat and marked with the installation of a 1.5 m long, 1.25 cm diameter polyvinyl chloride (PVC) pipe that was inserted vertically into the soil to resistance (approximately 0.3 m into the sand substrate) and recorded as a GPS waypoint (Garmin Montana 650, Garmin Ltd., United States). At one site in each cell, a 1.2 m long x 6 cm diameter piezometer (0.2 mm slits every 0.5 cm) was hammered into the soil to resistance and housed a continuous water level logger (model U20–04, OnSet Computer Corporation, Bourne, MA). Water level data was collected every 15 min throughout the study period. Initial (pre-WLDD) sampling occurred in both cells the week of November 26, 2019, then cell 11 was randomly chosen to receive the WLDD (henceforth refer to as the “treatment” cell) and cell 12 to serve as the “control” cell.

The WLDD began in the treatment cell in January 2020 and was



Fig. 1. Site map of the study location, the Orlando Easterly Wetland (OEW) in Christmas, FL, USA. Insert maps show regional location in the southeastern USA, while large map shows the configurations of wetland treatment cells within the OEW and the sampling areas.

accomplished by fully opening the treatment cell outflow while blocking the inflow weir with multiple wooden boards and a temporary earthen dam for a 15-month duration. To ensure the control cell continued to receive the same volume of inflow water as during normal operation, the total water volume to the southern flow-train was halved for the duration of the WLDD period. The naturally high groundwater table, absorptive properties of the soil, and rainfall limited the ability to achieve a subsurface water table through gravity drainage alone. Therefore, a canal was dug parallel to flow of the treatment cell in February 2020 and dewatering was enhanced with the use of a 15.24 cm diesel-powered hydraulic pump within the canal. Six field sampling dates included an initial/pre-WLDD (Nov. 26, 2019), four WLDD sampling dates: 3-months (March 4, 2020), 5-months (May 21, 2021), 9-months (Sept. 23, 2021), and 14-months (Feb. 20, 2021), and a post-WLDD sampling 2-month after reflooding (May 18, 2021). Reflooding began March 8, 2021, when the outflow weir was returned to operational height, the inflow blockage removed, and inflow water reintroduced to the treatment cell.

During each sampling date, all sites were visited within a 2-day period. Every time, the site PVC marker was approached from the NW to prevent soil disturbance on the remaining sides of the marker. Discrete surface water depth at the PVC pole was measured and recorded as the distance from the soil surface to the water surface. A surface water grab sample (100 mL) was collected in a Nalgene bottle for laboratory analysis of nutrients (nitrate + nitrite (henceforth reference to as NO_x), NH_4^+ , soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC)) if surface water was present. Basic surface water properties (temperature, pH, dissolved oxygen (DO), conductivity, and turbidity) were also recorded when possible, using a sonde (ProDSS, YSI, Inc., Yellow Springs, OH).

Soil elevation was documented as relative to the PVC marker pole installed at each site, which served as the reference post. During each sampling event, a 90° PVC coupling with a 0.5 m long PVC extension arm (parallel to the soil surface) was placed on the top of the reference post. A measuring stick was lowered vertically through the water column until it experienced the resistance of the soil surface, using care to avoid live vegetation. This measurement was repeated in each cardinal direction (4 measurements per site) and the 4 readings were averaged. Compaction of the soil adjacent to the reference post was prevented by maintaining a distance of at least 1 m from each post during all sampling activities.

A soil core (7 cm diameter x 2 m length clear polycarbonate tube with a beveled bottom edge) was collected at each site during every sampling event, between 1 and 5 m from the reference post. This core included both the floc layer and surface soil (0–5 cm) and was collected using the push-core method (hammering from above with a board and rubber mallet). To ensure no overlap with repeated samplings, coring began due N from the site PVC marker and moved ~22.5° clockwise each subsequent sampling (i.e., NE, E, SE, S, SW, W). Cores with disturbed floc layers where the floc/soil boundary was unidentifiable were discarded and recollected. The surface water (identified as translucent water with minimal particulate OM) was discarded in the field. Then, the floc layer was decanted from the top of the core with the assistance of a floc collar (fabricated from a plastic roofing vent boot and was affixed around the core after (Delaune et al., 2013) that guided the pouring of the floc into a gallon-sized Ziploc[®] freezer bag. The core extruder was demarcated by depth, allowed for the thickness of the floc layer to be determined as it was extruded. The boundary between the floc layer and soil was assessed through a field texture rub test and visually by the same person each time. The top 0–5 cm of soil was collected and stored in a Ziploc[®] freezer bag. All samples were placed on ice for transport to the lab.

To monitor surface water nutrients following reflooding (post-WLDD), grab samples were collected at the inflow and outflow weir(s) of both cells. Specifically, reflooding began March 8, 2021, but the treatment cell did not refill to overtop the outflow weir until March 14, 2021.

From March 14 to April 5, 2021 (first 22 days) surface water was collected daily. From April 5 to April 23, 2021, surface water was collected every other day for a total of 46 days of post-WLDD surface water sampling. Each time, water was collected with a 20 mL syringe submerged 5 cm below the surface of the water. Samples were field-filtered through a 0.45 μm membrane syringe filter into pre-acidified (<2 pH H_2SO_4) 20 mL scintillation vials and stored on ice during transport to the laboratory, and then at 4 °C until analysis for NO_x , NH_4^+ and SRP (see method below).

2.3. Laboratory experiment design and sample collection

Intact soil cores were collected from treatment cell 10 (Fig. 1), which was chosen due to the lack of previous cell renovations and its' central location within OEW. A total of 30 intact cores were collected within 40 m area (28° 34' 16.1328" N, 80° 59' 57.8904" W) with clear acrylic tubes (90 cm long x 7.54 cm diameter) using the push core method. All cores had at least 10 cm of soil OM above the underlying sand layer and no visible emergent vegetation. Five cores were immediately deconstructed for initial floc and soil physicochemical properties. Floc was transferred into gallon-sized polyethylene bags using the floc collar described above. The remaining 25 intact cores were transported to UCF packed in foam-filled crates to minimize disturbance. Depths of visible floc and sandy-mineral soil layers were measured and demarcated on the outside of the clear acrylic cores. Floating vegetation and any visible live fish or invertebrates were removed, and cores were allowed to acclimate for 1 week under flooded conditions; acclimatation allowed time for released labile C from severed roots and soil disturbance to be re-assimilated. All cores were sealed on the bottom with a vinyl endcap secured with multiple hose clamps, affixed in an upright position to a wire cage structure, and kept indoors in the dark at an ambient temperature (~20 °C). A fabricated PVC increaser was sealed to the top of each core to join the 7.54 cm diameter of the core tube to a 10.16 cm diameter ring, which allowed for an airtight connection to the 10 cm chamber of a LI-COR 8100 (LI-COR Biosciences, Lincoln, NE).

The 25 experimental cores were randomly assigned to one of 5 treatment conditions, where "WLDD" indicates a drop in the water table to -5 cm below the soil surface and "flood" indicates 50 cm of surface water, for the 57-d study. The treatments included: Flooded Control (57 d flood), Single Short (21 d WLDD, then 36 d flood), Multiple Short (14 d WLDD, 14 d flood, 14 d WLDD, and 14 d flood), Single Long (57 d WLDD), and Single Long Amended (20 g of native site clay was manually mixed into the top 10 cm of soil, then 57 d WLDD). During the experimental period, CO_2 flux was initially measured with the LI-COR twice daily (d 0–14), then once daily (d 15–28), and finally twice weekly (d 29–57). The total headspace volume, including the diameter increaser, chamber, and core, was measured and recorded for the LI-COR offset in the flux calculation. The order of measurement was randomized each time and a 5 min linear flux was used to calculate $\text{mg CO}_2\text{-C m}^{-2}\text{ d}^{-1}$. Flooded intact cores were refreshed 3 times a week with site water collected weekly from cell 10. To refresh, 250 mL of surface water from each core was carefully extracted with a syringe and hose. The apparatus was rinsed with DI water and then used to add 250 mL of fresh site water. At the conclusion of the intact core experiment, each core was destructively sampled by collecting the floc layer and the top 0–5 cm of soil. Samples were stored in gallon-sized polyethylene bags at 4 °C until analysis.

2.4. Soil and water physicochemical analysis

All soil cores (from both the field and laboratory experiments) were sectioned into floc and 0–5 cm of soil and analyzed for moisture content, bulk density, OM content, total N, P, and C, and extractable NO_x , NH_4^+ , SRP, and DOC. Moisture content was determined via gravimetric water content (i.e., drying a subsample at 70 °C until constant weight and quantifying mass loss) and dry bulk density was calculated using the

known mass and volume of soil collected. The dried sample was then placed in a 20 mL scintillation vial and ceramic balls were added to grind the sample to particle size on a SPEX Sample Prep 8000 M Mixer/Mill (SPEX, Metuchen, NJ, USA). A 5 mg subsample of this ground dried soil was then weighed in a tin capsule and analyzed for total C and N using an Elementar Vario Micro Cube (Elementar Americas Inc., Mount Laurel, NJ, USA). Another subsample (< 0.5 g) of dried, ground soil was combusted at 550 °C for 5 h in a muffle furnace to obtain OM content via loss on ignition (LOI) by change in weight. The remaining ash was then analyzed for total P by boiling in 25 mL of 1 M HCl on a hot plate for 30 min (Andersen, 1976). Once cooled, the liquid sample was filtered through Whatman #41 filter paper and analyzed on a Seal AQ2 Automated Discrete Analyzer via method 365.1 Rev. 2.0 (USEPA, 1993).

Extractable NO_x , NH_4^+ , SRP, and DOC were extracted within 24 h of collection on field wet samples. First, 3–4 g of homogenized soil was placed in a 40 mL centrifuge tube and 25 mL of 2 M KCl was added. Samples were shaken on an orbital shaker at 150 rpm at 25 °C for 1 h, then centrifuged at 5000 rpm at 10 °C for 10 min. The supernatant was filtered through a Supor 0.45 μm filter (Pall Corporation, Port Washington, NY, USA), acidified to a pH <2 with double distilled H_2SO_4 , and stored at 4 °C. Analysis for NO_x , NH_4^+ , SRP was performed on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI, USA) using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively (USEPA, 1993). A Shimadzu TOC-L Analyzer (Shimadzu Scientific Instruments, Kyoto, Japan) was used to determine the concentration of non-purgeable DOC in the water samples. Surface water samples from the post-reflooding phase of the field experiment were analyzed in the same manner as the soil extractable NO_x , NH_4^+ , SRP, and DOC, but without the KCl extraction procedure and used a freshwater analytical matrix instead of a KCl matrix.

2.5. Soil organic matter fractionation

Following destructive sampling of the laboratory intact soil core experiment, samples from the Single Long Amended and Flooded Control were physically- and density fractionated to quantify mineral associated organic matter (MAOM) following recommendations for wetland soils after Mirabito and Chambers (2023). Briefly, 20 g dry-equivalent of field moist soil was weighed and 0.5 % sodium hexametaphosphate added to create a slurry (1:8 soil to solution ratio). Samples shook for 18 h at 150 RPM on an orbital shaker. Next, samples were wet sieved using 2 mm, 250 μm and a 53 μm sieves and oven dried at 70 °C until constant weight. Soil in the fraction <53 μm was then density fractionated using 1.85 g cm^{-3} sodium polytungstate (SPT). First, 25 mL of SPT solution was added to 2 g of dry soil and shaken for 18 h at 150 RPM on an orbital shaker. Additional SPT solution was added to rinse off soil and then centrifuge at 5000 RPM for 30 min at 20 °C. Any light fraction (LF) was decanted onto a 0.45 μm filter and was rinsed of SPT solution and backwashed into a pre-weighed container. Centrifuging and filtering was repeated once again with SPT solution, and then with deionized water. The heavy fraction (HF) was backwashed into a pre-weighed container and all samples were oven dried at 70 °C until constant weight. All soils were ground until fully homogenized using ceramic balls in a SPEX 8000 M Mixer/Mill (SPEX Sample Prep, Metuchen, NJ, USA) then analyzed for total C and N as described above.

2.6. Data analysis

All statistical analyses were performed using R Statistical Software stats package (v.4.3.0; R Core Team, 2023) within Rstudio IDE (v2023.3.0.386; Posit Team, 2023). All data wrangling was completed using Tidyverse R Package (v2.0.0; Wickham et al., 2019) and data visuals were created with ggplot2 R package (v3.4.2; Wickham, 2016) and Microsoft Excel (2016). In the field study, the variances between cells were not assumed to be equal and a Welch's two sample *t*-test was used. The alpha level for all statistical analysis was set at (α) = 0.05. All

residuals were visually assessed for normality. The Welch’s *t*-test was used to identify differences between the treatment and control cells per sampling event for the following: water level, soil elevation, floc thickness, and all soil physicochemical properties of floc and soil. For water level, soil elevation, and floc thickness, Welch’s *t*-tests were run for each of the 6 sampling events, whereas for physiochemical properties the 6 sampling events were grouped into one of four of the following: pre-WLDD, dry event #1 and #2, and post-WLDD. The treatment cell data pre- and post- WLDD was compared with Welch’s two sample *t*-test. Some data sets (bulk density, extractable NO_x, NH₄⁺, and SRP) were log transformed prior to analysis to meet the assumption of normality.

For the lab study, one-way ANOVA models were used to determine differences by treatment for the following: mg CO₂-C flux rate, floc thickness, soil elevation, and all physicochemical properties. A post-hoc Tukey’s HSD test for multiple comparisons was used for each ANOVA where the alpha level was met. Assumptions of normality and homogeneity of variance were confirmed met by visually assessing the model residuals. For both the field and lab study, outliers were removed with the Interquartile Range (IQR) test that were greater or <1.5 * IQR ± mean.

For soil organic matter fractionation, normality was checked visually using both histograms and Q-Q plots and statistically using the Shapiro Wilk’s test (*p* > 0.05), and homogeneity was determined by Levene’s test (*p* > 0.05). Outliers were removed by the Interquartile Range (IQR) test which removed samples that were greater or <1.5 * IQR. For both the total C and N, generalized linear models (GLM) were used (parameter ~ treatment * size fraction) both using a Gamma distribution. The best distribution for the GLM was determined by using the *fitdistrplus* R

package (Delignette-Muller and Dutang, 2015). When several distributions fit the data, the model with the lowest AICc score was chosen. Following the GLM, post hoc testing using a Tukey adjustment was done using the *emmeans* package (Lenth, 2020).

3. Results

3.1. Field experiment: Floc thickness, water and soil depth

Mean surface water depth in the control cell ranged from 63 ± 4 to 82 ± 4 cm (mean ± standard error) during the experiment, based on discrete measurements during sampling (Fig. 2). Pre- and post- WLDD, surface water depth in the treatment cell did not differ from the control cell. The WLDD (i.e., sampling events 2–5 when the inflow to the treatment cell was blocked-off) occurred from January 2020–April 2021, but full dry-down (i.e., average water level within ±3 cm of the soil surface with locations of dry, cracked soil) was only achieved for two sampling events, May 2020 (dry event #1) and February 2021 (dry event #2; Fig. 2a). Based on the continuous water level logger data, dry event #1 lasted 22 days with samples collected on day 15, and dry event #2 lasted 20 days with samples collected on day 18 (Supp. Fig. 1). The March 2020 and Sept. 2021 sampling events had lower water levels in the treatment cell than the control cell but remained shallowly inundated with rainwater.

Soil elevation averaged 6.8 cm higher in the control cell (116.0 ± 1.8 cm) than the treatment cell (109.1 ± 2.2 cm) at the initial (pre-WLDD) sampling event, due to the difference in depth the reference poles were installed into the soil. Therefore, soil elevation change

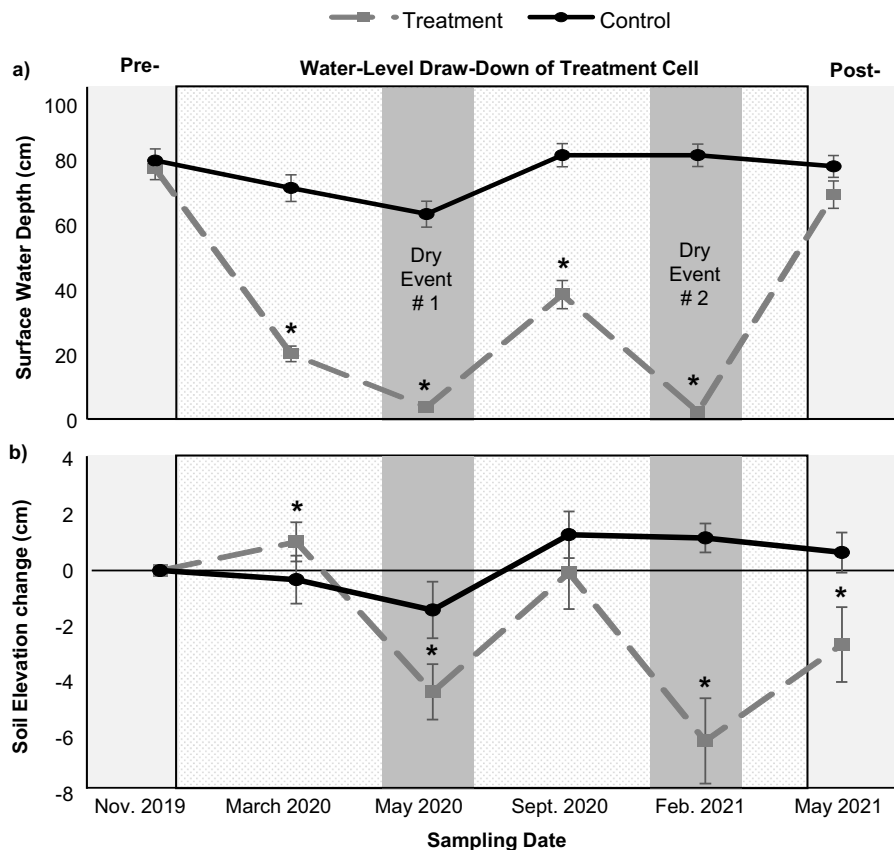


Fig. 2. Discrete surface water depth (a) and change in soil elevation from the pre-water-level dry-down (WLDD) condition (b) for the treatment and control wetland cells used in the field experiment by sampling date. The gray dot-textured box represents the period of time the treatment cell inflow was stopped to initiate WLDD, while the medium gray columns indicate when each dry event (#1 and #2) was achieved (i.e., average water level below the soil surface in the treatment cell). Data are mean ± standard error; *n* = 14. Asterisks indicate significant differences (*p* < 0.05) between the treatment and control cells by sampling date based on a Welch’s *t*-test.

(difference from initial) was used to quantify the effect of the WLDD on soil elevation in each cell. As the water level was first reduced, soil elevation in the treatment cell increased relative to the control cell ($t = 2.4$, $p = 0.02$; Fig. 2b). The two dry events resulted in the largest change in soil elevation in the treatment cell, -4.34 ± 0.84 cm in dry event #1 ($t = -4.1$, $p < 0.001$) and -5.90 ± 1.54 cm in dry event #2 ($t = -8.7$, $p < 0.001$). During the partial rainwater reflooding of the treatment cell in Sept 2020, mean soil elevation rebounded to be equivalent to the initial elevation of 0.0 ± 5.04 cm. However, a significant change in soil elevation in the treatment cell persisted post-WLDD on May 2021, with a -2.66 ± 1.38 cm change (Fig. 2b). During the post-WLDD sampling, the treatment cell soil elevation was both lower than the control cell ($t = -4.3$, $p < 0.001$) and lower than the pre-WLDD elevation of the treatment cell itself ($t = 4.9$, $p < 0.001$). Throughout the study period, the soil elevation in the control cell fluctuated slightly between 1.2 and -1.4 cm of the initial measurement.

The thicknesses of the floc layers in both cells were 24.9 ± 2.6 cm for the initial (pre-WLDD) sampling (Fig. 3). Following initiation of the WLDD, average floc thickness was lower in the treatment cell, relative to the control cell, for every subsequent sampling date (averaging 8.8 ± 2.2 and 27.4 ± 1.7 cm, respectively; all $p \leq 0.001$). This difference in floc thickness between cells persisted in the final post-WLDD sampling (treatment cell = 10.6 ± 1.2 cm; control cell = 24.3 ± 4.1 cm; $t = -6.99$, $p < 0.001$). Overall, floc thickness in the treatment cell decreased an average of 16.4 cm, or 60.6 % lower than initial, which was a significant decline from pre- to post-WLDD ($t = 9.3$, $p < 0.001$). WLDD also substantially reduced the variability in floc thickness between sample points. For example, the average interquartile range (IQR) for the treatment cell of only 4.6 ± 1.2 cm at the end of the experiment, as compared to 22.8 ± 1.9 cm for the control cell (Fig. 3).

3.2. Field experiment: Floc and soil physicochemical properties

Pre-WLDD, floc had similar physicochemical properties across both cells, with a moisture content of 98.5 ± 0.11 %, bulk density of 0.013 ± 0.001 g cm⁻³, and OM content of 75.0 ± 2.2 %. Dry event #1 resulted in a 24.4 % reduction in the average floc moisture content and a 4× increase in the average floc bulk density in the treatment cell, relative to the control cell, but OM content remained similar between cells (Table 1). By dry event #2, all three floc physical properties differed by cell (Table 1). These significant cell differences persisted after reflooding

the treatment cell (post-WLDD), though the magnitude of the differences was less extreme than observed during the dry events themselves. Specifically, floc averaged a 2.6 % lower moisture content, a 140 % higher bulk density, and a 12.7 % lower OM content in the treatment cell, relative to the control cell, post-WLDD. Within the treatment cell itself, this represented a significant decrease in floc moisture content ($t = 5.5$, $p < 0.001$) and increase in floc bulk density ($t = -6.2$, $p < 0.001$) between pre- and post-WLDD.

The WLDD had less impact on surface soil (0–5 cm) physicochemical properties. Prior to the experiment (pre-WLDD), both cells had a soil moisture content of 38.3 ± 2.8 and a bulk density of 1.024 ± 0.066 g cm⁻³; soil OM varied slightly by cell (2.69 ± 0.38 and 4.52 ± 0.55 % for the treatment and control cells, respectively; Table 1). Throughout the remainder of the experiment, moisture content and OM content did not differ by cell and bulk density only differed during the final post-WLDD sampling. Specifically, soil bulk density averaged 33 % greater in the treatment cell, relative to the control cell, after reflooding. Within the treatment cell, there were no significant differences in soil physicochemical properties between pre- and post-WLDD.

Pre-WLDD, average extractable N and P concentrations across both cells were 47, 24, and 34 times higher in the floc layer than the soil (0–5 cm) layer, for NO_x, NH₄⁺, and SRP, respectively. Despite starting and ending with comparable extractable nutrient concentration in the treatment and control cells, all three nutrients showed some differences between cells during one or both dry events. Floc extractable NO_x increased 40-fold during dry event #1 and 16-fold during dry event #2 in the treatment cell, relative to the control (Table 1). A similar trend was observed for soil extractable NO_x, which increased 64-fold during dry event #1 and 20-fold during dry event #2 in the treatment cell, relative to the control. Extractable NH₄⁺ also increased in the treatment cell during dry event #1 (a 2.3-fold increase in the floc and a 1.5-fold increase in the soil). However, during dry event #2 the relationship flipped—the extractable NH₄⁺ was 2.6-fold lower in floc and 1.6-fold lower in the soil of the treatment cell, relative to the control cell. Finally, extractable SRP showed no effect of the first dry-down, but approximately doubled in both the floc and soil of the treatment cell, relative to the control cell, during dry event #2 (Table 1). Unlike extractable N and P, extractable DOC did not differ significantly between the floc and soil initially and was even slightly higher in the soil (Table 1). However, DOC increased 8-fold and 3.6-fold in the treatment cell floc layer during dry events #1 and #2, respectively, before

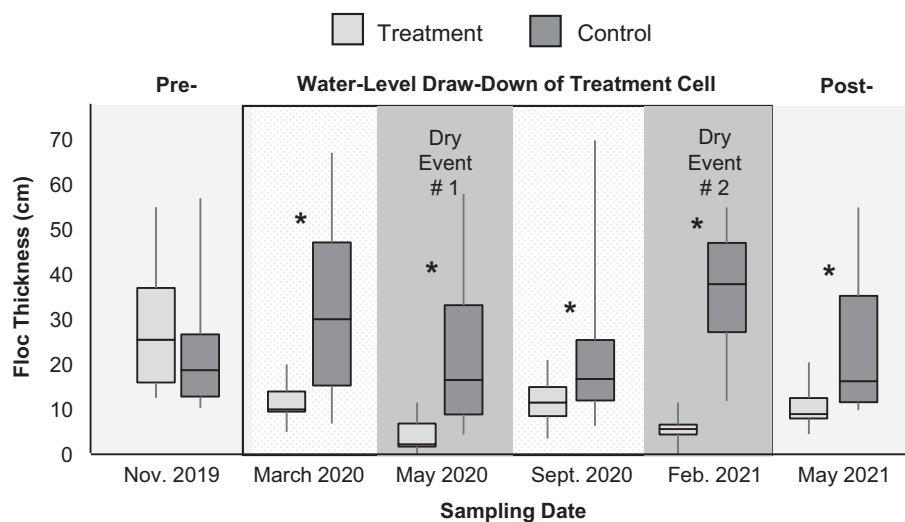


Fig. 3. Floc thickness for the treatment and control wetland cells used in the field experiment by sampling event. The gray dot-textured box represents the period of time the treatment cell inflow was stopped to initiate water-level dry-down, while the medium gray columns indicate when each dry event (#1 and #2) was achieved (i.e., average water level below the soil surface in the treatment cell). Boxes are median and 1st and 3rd quartiles, while whiskers represent max and min values; $n = 14$. Asterisks indicate significant differences ($p < 0.05$) between the treatment and control cells by sampling date based on a Welch's t-test.

Table 1

Soil physicochemical properties (mean \pm standard error; $n = 14$) in the two experimental field wetland cells: the treatment cell (treat.), which received the water-level dry-down (WLDD), and the reference (control) cell. Data is presented for the key sampling dates of interest: initial (pre-WLDD), the two sampling dates where average water-level dropped below the soil surface in the treatment cell (dry events #1 and #2), and after reflooding the treatment cell (post-WLDD). Data is presented for the floc layer (F) and surface (0–5 cm) soil (S). Based on a Welch's t-test comparing the treatment and control cells at each sampling date, merged columns represent no significant difference between cells ($p > 0.05$), separated columns represent differences with a $p < 0.05$ to >0.01 ; bold represents $p \leq 0.01$ to >0.001 ; bold italics represents $p < 0.001$.

Cell		Pre-WLDD		Dry Event #1		Dry Event #2		Post-WLDD	
		(Nov. 2019)		(May 2020)		(Feb. 2021)		(May 2021)	
		Treat.	Control	Treat.	Control	Treat.	Control	Treat.	Control
Moist. Cont. (%)	F	98.5 \pm 0.11		72.9 \pm 4.6	97.3 \pm 0.4	83.2 \pm 2.1	98.3 \pm 0.1	96.0 \pm 0.4	98.6 \pm 0.1
	S	38.8 \pm 2.8		38.0 \pm 2.0		31.8 \pm 1.5		35.2 \pm 2.4	
Bulk Density ($g\ cm^{-3}$)	F	0.013 \pm 0.001		0.093 \pm 0.014	0.023 \pm 0.003	0.091 \pm 0.011	0.017 \pm 0.002	0.034 \pm 0.004	0.014 \pm 0.002
	S	1.024 \pm 0.066		0.960 \pm 0.048		1.148 \pm 0.049		1.296 \pm 0.064	0.974 \pm 0.117
OM (%)	F	75.0 \pm 2.2		70.2 \pm 3.3		57.0 \pm 4.7	71.2 \pm 3.3	72.5 \pm 3.1	83.1 \pm 2.0
	S	2.69 \pm 0.38	4.52 \pm 0.55	5.68 \pm 0.79		3.42 \pm 0.35		3.17 \pm 0.44	
Extract. NO _x ($mg\ L^{-1}$)	F	46.8 \pm 4.0		137.8 \pm 29.9	3.49 \pm 0.60	192.9 \pm 53.5	11.7 \pm 1.71	8.09 \pm 1.33	
	S	1.00 \pm 0.07		9.22 \pm 3.41	0.14 \pm 0.01	8.16 \pm 2.21	0.40 \pm 0.03	0.25 \pm 0.03	
Extract. NH ₄ ⁺ ($mg\ L^{-1}$)	F	88.5 \pm 19.6		152.9 \pm 44.1	67.8 \pm 11.4	38.3 \pm 10.0	99.5 \pm 6.16	87.6 \pm 11.0	
	S	3.62 \pm 0.85		4.77 \pm 1.65	3.12 \pm 0.77	1.91 \pm 0.30	3.13 \pm 0.25	3.77 \pm 0.40	
Extract. SRP ($mg\ L^{-1}$)	F	52.7 \pm 10.5		53.8 \pm 8.8		64.1 \pm 15.2	23.0 \pm 5.31	18.5 \pm 2.78	
	S	1.56 \pm 0.21		2.11 \pm 0.34		2.30 \pm 0.44	0.88 \pm 0.17	0.94 \pm 0.16	
Extract. DOC ($mg\ L^{-1}$)	F	1.90 \pm 0.17		11.2 \pm 2.1	1.42 \pm 0.20	6.36 \pm 0.56	1.76 \pm 0.12	5.92 \pm 1.42	
	S	2.99 \pm 0.43		1.81 \pm 0.12		2.08 \pm 0.15		1.83 \pm 0.13	

returning to concentrations comparable to the control cell floc post-WLDD. The WLDD had no effect on soil DOC.

Pre-WLDD, total C averaged 388.2 \pm 11.3 mg kg⁻¹ in the floc and 28.6 \pm 0.3 mg kg⁻¹ in the soil for both cells. No difference in total C was observed between cells except during dry event #2 when the treatment cell floc decreased (309.4 \pm 23.3 mg C kg⁻¹) relative to the control cell floc (369.2 \pm 15.6 mg C kg⁻¹; $t = -2.23$, $p = 0.04$). Post-WLDD, total C was again similar between cells (377.6 \pm 11.9 mg C kg⁻¹ in the floc and 17.8 \pm 3.6 mg C kg⁻¹ in the soil). Total N was slightly greater in the floc of the treatment cell during the pre-WLDD and dry event #1 (28.8 \pm 14.8 and 28.2 \pm 18.6 mg N kg⁻¹, respectively) compared to the control cell (24.2 \pm 17.4 and 21.9 \pm 19.1 mg N kg⁻¹, respectively; $t = 2.12$, $p = 0.04$ and $t = 2.25$, $p = 0.01$), but was otherwise similar throughout the study. No treatment effect was observed in soil total N, which averaged 1.46 \pm 0.12 mg N kg⁻¹ in both cells. Total P began with similar concentrations between cells for floc (115.6 \pm 4.5 mg P kg⁻¹), diverged during the first dry event (121.4 \pm 15.8 mg P kg⁻¹ in the treatment cell, compared to 78.1 \pm 7.21 mg P kg⁻¹ in the control cell; $t = 2.31$, $p = 0.03$), then remain comparable for all other sampling events. The soil had higher total P in the control cell (6.73 \pm 1.41 mg P kg⁻¹) relative to the treatment cell (2.93 \pm 0.42 mg P kg⁻¹; $t = -3.27$, $p = 0.003$) pre-WLDD, but were similar throughout the remainder of the study.

3.3. Field experiment: reflooding nutrient dynamics

Surface water nutrient concentrations were measured at the common inflow to both cells, the outflow of the treatment cell, and the outflow of the control cell for a 46-day period that began on March 8, 2021, the day the treatment cell inflow was re-opened to begin reflooding. During this period, inflow NO_x was 0.91 \pm 7.21 mg L⁻¹, while outflow NO_x in both cells remained similar at 0.01 \pm 0.004 mg L⁻¹, representing a ~ 99 % decline in NO_x and no impact from the WLDD (Fig. 4a). Concentrations of NH₄⁺ were similar at all 3 sampling locations, averaging 0.10 \pm 0.01 mg L⁻¹, with no measurable removal from inflow to outflow and no effect from the WLDD (Fig. 4b). On the contrary, SRP removal averaged ~ 37 % in the control cell (inflow SRP = 0.19 \pm 0.03 mg L⁻¹ and control cell outflow SRP = 0.12 \pm 0.01 mg L⁻¹) but showed a temporary release in the treatment cell (Fig. 4c). Treatment cell SRP spiked to a maximum of 1.5 mg L⁻¹ immediately after reflooding, then slowly declined to match control cell outflow concentrations by day 36.

3.4. Laboratory experiment: CO₂ flux

All intact soil cores began flooded and had comparable CO₂ flux rates (404 \pm 44 mg CO₂-C m⁻² day⁻¹ on day -1; Fig. 5a, b). While the Flooded Control continued to produce similar rates for the remainder of the 57-d experiment (448 \pm 15 mg CO₂-C m⁻² day⁻¹), WLDD had a rapid and significant effect on all other treatments. Except for the Single Long Amended cores, average fluxes for all other cores when dry were 2423 \pm 52 mg CO₂-C m⁻² day⁻¹, which was 4.8 times greater than the flux rate observed when flooded (502 \pm 15 mg CO₂-C m⁻² day⁻¹). Cumulative C loss showed a positive linear relationship to the total length of the WLDD among all non-amended cores ($y = 0.0074x$, $R^2 = 0.93$). Specifically, the Single Long (57 d WLDD) released the most CO₂-C (6,917 \pm 756 g), which was greater than the Multiple Short (3797 \pm 301 g; 28 d WLDD), Single Short (3210 \pm 335 g; 21 d WLDD), and Flooded Control (1200 \pm 124 g; 0 d WLDD; all $p < 0.001$; Fig. 5c). The Single Long Amended treatment also had a 57 d WLDD, but total CO₂-C loss was 36 % less than the Single Long without the clay amendment (4408 \pm 583 g; $p < 0.001$), making the C flux comparable to the Single and Multiple Short treatments (Fig. 5c).

3.5. Laboratory experiment: Floc and soil physical properties

Initial floc thickness in all intact cores averaged 52.9 \pm 1.4 cm. By the conclusion of the experiment, all treatments except the Flooded Control had a significant decrease in floc thickness (Fig. 6a). Specifically, the floc thickness in the Single Long Amended treatment decreased the most (-22 cm; a 41 % reduction), but was not significantly greater than the Single Long, Single Short, and Multiple Short (all approximately -19 cm, representing a 36 % reduction from initial floc thickness). Meanwhile, the bulk density of the floc layer increased in all treatments after the laboratory core experiment (all $p \leq 0.04$) when compared to initial bulk density measurements (Fig. 6b). Between treatments, final floc bulk density was greater in the Single Long ($0.88 \times 10^{-3} \pm 0.009 \times 10^{-3}$ g cm⁻³) and Single Long Amended ($1.1 \times 10^{-3} \pm 0.14 \times 10^{-3}$ g cm⁻³) cores when compared to the Control cores ($0.47 \times 10^{-3} \pm 0.08 \times 10^{-3}$ g cm⁻³). Based on the observed increase in floc bulk density (as compared to the initial cores) and the decrease in floc thickness (as compared to day -1 of the experiment), the contribution of oxidation to the decrease in floc thickness was calculated to be 4.4 \pm 0.4 % for all treatments exposed to some dry-down (i.e., excluding the Control treatment) and did not differ with treatment. This leaves the

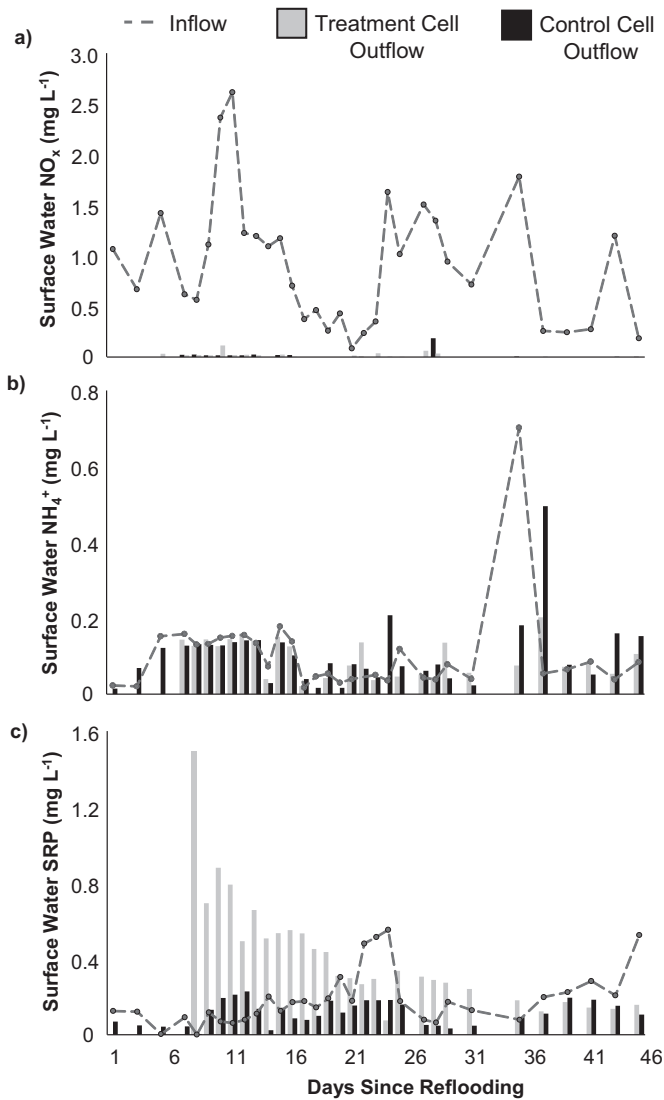


Fig. 4. Surface water nutrient concentrations from samples collected at the inflow (dashed line) of the two experimental wetland cells, the outflow of the treatment (WLDD) cell (gray bars), and the outflow of the control cell (black bars). Data are discrete measurements of a) nitrate+nitrite (NO_x), b) ammonium (NH_4^+), and c) soluble reactive phosphorus (SRP) for the first 46 days after reflooding of the treatment cell.

remaining 95.6 ± 0.4 % of the loss in floc thickness attributable to compaction.

Mean soil elevation remained unchanged in the Control treatment but decreased slightly (though not significantly) across all other treatments. The largest decrease in soil elevation was observed in the Single Short and Single Long Amended treatments, averaging 0.6 and 0.5 cm, respectively. Soil bulk density also did not change during the experiment under any conditions, averaging $0.49 \pm 0.05 \text{ g cm}^{-3}$.

3.6. Laboratory experiment: Floc and soil biogeochemical properties

The OM content of the Single Long Amended floc decreased during the study, ending with 60.3 ± 6.7 % OM, relative to the 84.2 ± 2.6 % OM of the initial cores (Fig. 6c). Floc total C ($388 \pm 15 \text{ g kg}^{-1}$), total N ($25.8 \pm 1.0 \text{ g kg}^{-1}$) and total P ($1.27 \pm 0.05 \text{ g kg}^{-1}$) did not change from initial conditions for any treatments. Soil OM (20.6 ± 3.5 %), total C ($168 \pm 24 \text{ g kg}^{-1}$) and total N ($11.4 \pm 1.5 \text{ g kg}^{-1}$) were also unaffected by time or treatment. However, soil total P did increase in all treatments

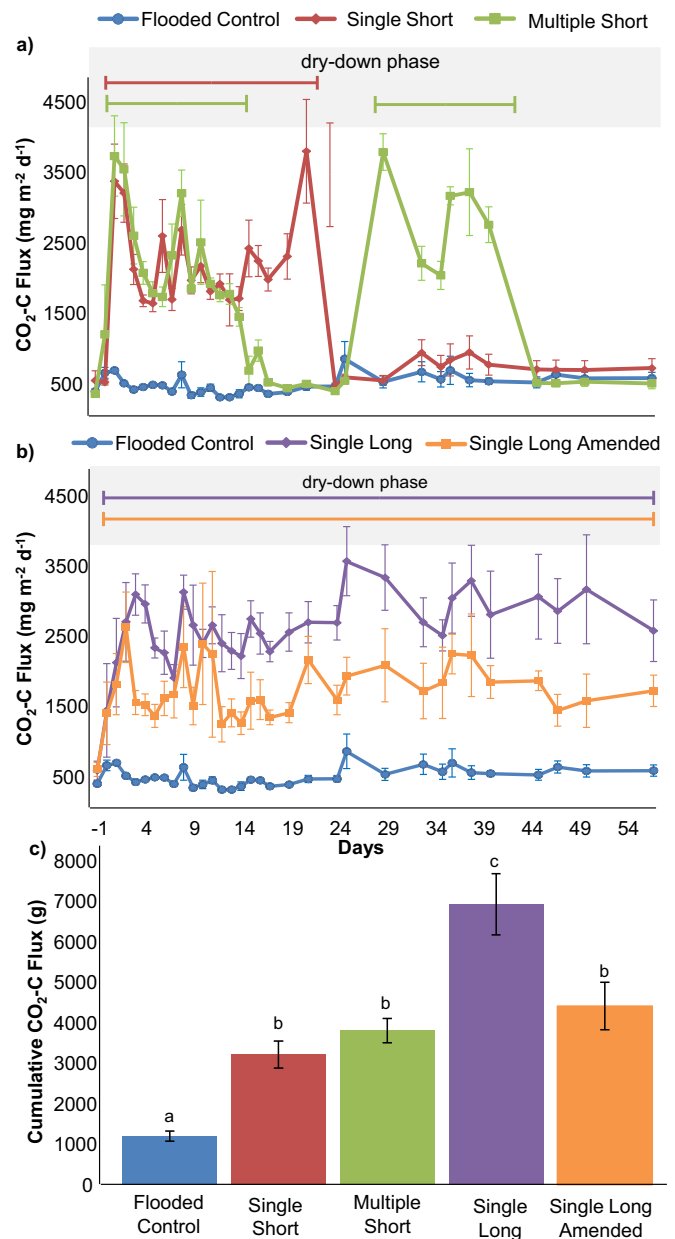


Fig. 5. Flux rate of $\text{CO}_2\text{-C}$ from intact soil cores by treatment over the 57-day experiment. Each point represents mean \pm standard error ($n = 5$). For ease of viewing, Short WLDD treatments are presented in panel (a), while Long WLDD treatments are present in panel (b); the Flooded Control is presented in both for comparison. Cumulative flux for all treatments is presented in panel (c) with different letters representing significant difference ($p < 0.001$) according to a Tukey's HSD.

during the experiment from an initial concentration of $0.05 \pm 0.01 \text{ g kg}^{-1}$ to a final average of $0.28 \pm 0.0 \text{ g kg}^{-1}$ across treatments.

The distribution of total C and N among soil (0–5 cm) size fractions was only evaluated in the Single Long Amended and Single Long (un-amended) treatments to specifically evaluate the impact of the clay amendment. Total C in the largest size fraction ($> 2 \text{ mm}$) was greater in the Single Long Amended treatment ($30.83 \pm 14.12 \text{ g kg}^{-1}$) compared to the un-amended treatment ($5.88 \pm 1.77 \text{ g kg}^{-1}$; $p = 0.022$). In the smallest size fraction ($< 53 \mu\text{m}$), there was more than twice as much total C in HF (i.e., MAOM pool) in the Single Long Amended treatment ($1.88 \pm 0.71 \text{ g kg}^{-1}$) compared to the un-amended treatment ($0.88 \pm 0.06 \text{ g kg}^{-1}$) and in the $< 53 \mu\text{m}$ LF of the Amended treatment ($32.19 \pm 11.14 \text{ g kg}^{-1}$) compared to the unamended ($11.13 \pm 0.89 \text{ g kg}^{-1}$), but these

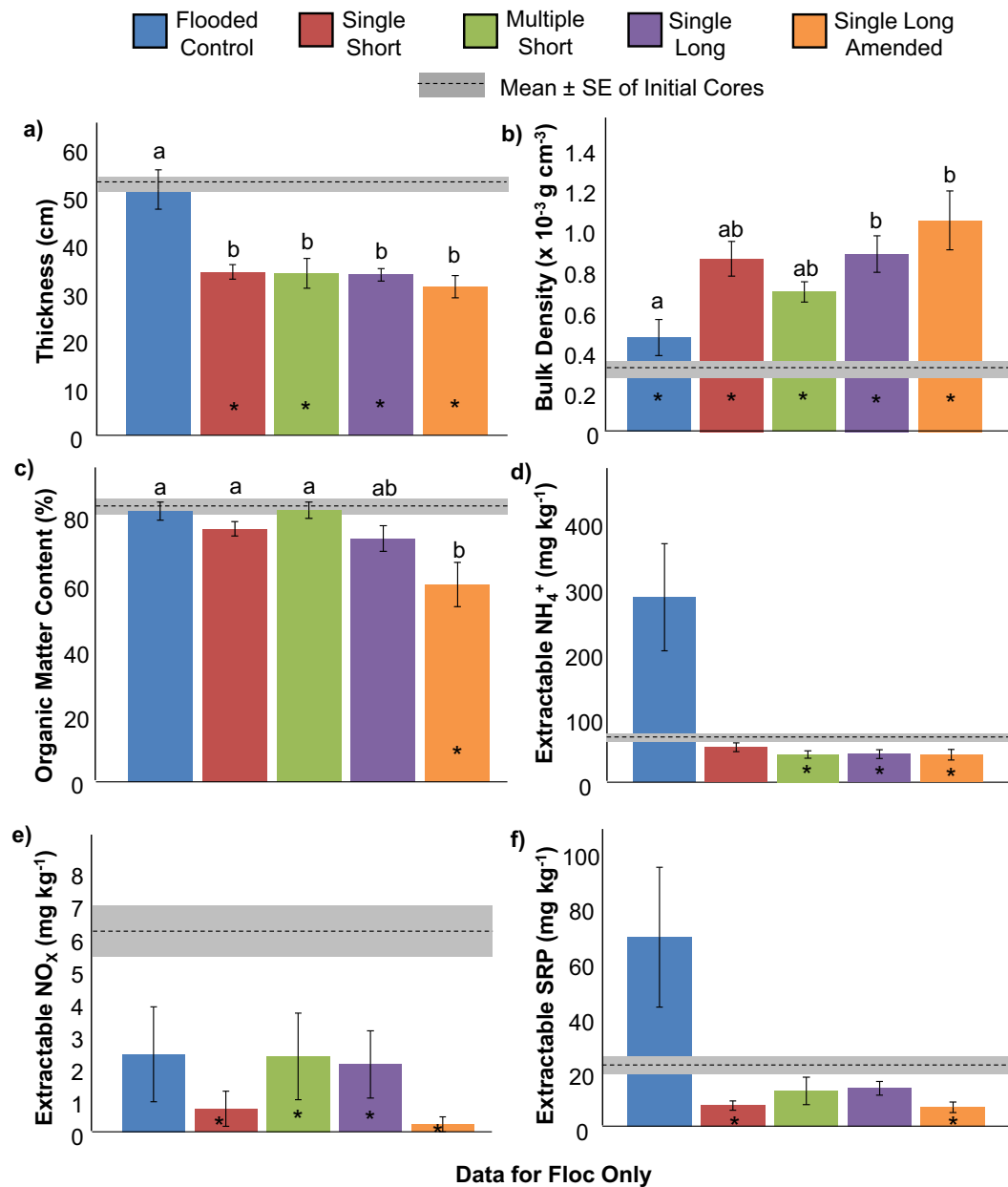


Fig. 6. Floc physicochemical and biogeochemical properties from the intact core experiment (mean \pm standard error (SE)), presented by treatment ($n = 5$ for each). Colored vertical bars are data from soil cores destructively sampled after the conclusion of the 57-day experiment (post- or ‘after’ conditions), while gray horizontal bars are data from initial soil cores (pre- or ‘before’ conditions) destructively sampled at the beginning of the experiment. Asterisks represent significant differences ($p \leq 0.05$) between the pre- and post- data based on a Welch’s t-test. Different letters represent significant differences ($p \leq 0.05$) between different treatments post-experiment based on a Tukey’s HSD.

differences were not significant ($p = 0.18$ and 0.11 , respectively). For total N, there was a significant size fraction \times treatment effect ($F = 3.35$, $p = 0.021$). Similar to total C, total N in the >2 mm size fraction was greater in the Single Long Amended treatment (1.89 ± 0.91 g kg⁻¹) compared to the un-amended (0.98 ± 0.90 g kg⁻¹; $p = 0.020$) and MAOM total N averaged six-times greater in the Single Long Amended treatment (0.12 ± 0.05 g kg⁻¹) than the un-amended (0.02 ± 0.01 g kg⁻¹; $p = 0.013$). The <53 μ m LF also generally had more total N in the Single Long Amended treatment (2.5 ± 0.90 g kg⁻¹) compared to the Single Long (1.44 ± 0.57 g kg⁻¹).

In the floc, extractable NH₄⁺ and SRP generally decreased relative to the Initial core concentrations in all treatments except the Control, where it tended to increase (Fig. 6d and f). For example, extractable NH₄⁺ in the Multiple Short, Single Long, and Single Long Amended cores

averaged 42.6 ± 3.7 mg kg⁻¹ after the experiment, which was less than the Initial cores (69.6 ± 6.4 mg kg⁻¹; all $p \leq 0.03$). Meanwhile, the Control tended to increase (238.8 ± 82.1 mg kg⁻¹; $p = 0.06$) in extractable NH₄⁺. Extractable SRP decreased in the Single Short (7.7 ± 1.7 mg kg⁻¹) and Single Long Amended (7.14 ± 1.9 mg kg⁻¹) treatments relative to the Initial cores (22.4 ± 3.1 mg kg⁻¹; $p \leq 0.01$), while the Control tended to increase (68.9 ± 25.4 mg kg⁻¹; $p = 0.1$). All treatments averaged lower extractable NO_x in the floc after the experiment when compared to the Initial cores (6.2 ± 0.8 mg kg⁻¹; Fig. 6e). This decrease in extractable NO_x was most pronounced in the Single Short (0.7 ± 0.5 mg kg⁻¹; $p = 0.001$) and Single Long Amended (0.2 ± 0.2 mg kg⁻¹; $p < 0.001$) treatments. Looking at overall dissolved inorganic N (DIN = NH₄⁺ + NO_x) dynamics in the floc, Multiple Short, Single Long, and Single Long Amended cores had a similar ~ 41 – 44 % reduction in

DIN due to the treatment conditions, while the Single Short reduced DIN ~18 %; the flooded Control increased DIN by ~278 % relative to Initial core concentrations (data not shown).

Trends in soil extractable nutrients mirrored that of the floc data for NH_4^+ and SRP, with all WLDD treatments generally decreasing relative to the Initial core concentrations while the Control treatment increased. Within-treatment variability limited the statistical significance in the soil trends, but average soil extractable NH_4^+ decreased ~56–78 % in all WLDD treatments while increasing by ~79 % in the Control relative to the Initial cores. Likewise, average soil extractable SRP decreased ~80–92 % in all WLDD treatments but increased ~76 % in the Control. Also mirroring the floc results, soil extractable NO_x consistently decreased relative to the Initial cores across all treatments. The decrease was significant in all WLDD cores (averaging $0.08 \pm 0.04 \text{ mg kg}^{-1}$, or a ~86–100 % reduction) compared to the Initial cores ($1.3 \pm 0.2 \text{ mg kg}^{-1}$; all $p \leq 0.05$), and also generally decreased in the Control ($0.8 \pm 0.5 \text{ mg kg}^{-1}$). Overall, average soil DIN reduction was similar in the Single Short, Multiple Short, and Single Long treatments at ~73–78 % removal, an approximately 58 % reduction in the Single Long Amended treatment, and a 74 % increase in DIN for the Control, relative to Initial core concentrations.

4. Discussion

4.1. Water level draw-down effectively reduced floc and soil via consolidation

The greatest volume of OM likely obstructing sheet flow in this CTW was not associated with the soil, but rather the floc (unconsolidated, minimally decomposed, organic detritus suspended in the water column). Other CTWs have identified the presence of a floc layer, but the thickness of the floc observed at OEW was unique. For example, the floc layer in a South Carolina, USA CTW was 0–2 cm after 4 y (0–7 % of the water column depth; Knox et al., 2010) and another CTW in central FL, USA had 5–11 cm of floc after 12 y (15 % of the water column depth; Zamorano et al., 2018). By comparison, the average floc layer in the OEW study cells was 27 cm, but up to 70 cm in some areas, occupying 35–92 % of the water column depth after 10–12 y. This low-density floc was predominately water-filled interstitial space (only ~1.5 % solids) and was a consequence of the combination of high primary production and the lack of hydrologic variability (i.e., continuous flooding with $75.8 \pm 3.0 \text{ cm}$ of surface water). Prior research shows that regardless of the quantity of total suspended solids in the water column, these suspended materials are not retained or incorporated into the soil unless desiccation (dry-down) occasionally occurs (Day et al., 2011). When the water table drops below the soil surface, organic fibers contract/shrink as they dry and the interstitial spaces previously filled with water are replaced by gas, allowing gravity to collapse and consolidate the solids, and thus increasing the soil bulk density through the loss of volume (Chambers et al., 2019; Hooijer et al., 2012).

As the water level in the treatment cell began to drop, the average floc thickness rapidly decreased (by approximately 56 % with partial drying (March 2020) and 85 % with full drying (May 2020)). Likewise, the moisture content of the floc dropped from roughly 98.5 % pre-WLDD, to 73–83 % during the dry events, and the floc bulk density increased 7-fold (Table 1). An initial increase of ~1 cm in average soil elevation at the first WLDD sampling (March 2020) suggests the floc first settled on the soil surface. Then, further water table drop below the soil surface caused both floc and soil consolidation, decreasing the soil elevation an average of 4.3 cm and 6.1 cm (dry events #1 and #2, respectively) below the initial elevation. Although some re-expansion of the floc and soil OM was observed upon re-wetting (i.e., post-WLDD re-flooding), both floc thickness and soil elevation remained less than both its' pre-WLDD condition, and below that of the control cell. On average, floc thickness was permanently reduced by 60 % and soil elevation was reduced by 2.7 cm in the treatment cell. No published data was found to

compare floc thickness change to previous studies, but a significant amount of data is available on the rate of soil elevation loss due to drainage in peatlands, most of which demonstrates a linear relationship with water table depth (Couwenberg et al., 2010; Hooijer et al., 2012). Consensus from peatland studies indicates an initial (e.g., first 1–2 years post-drainage) rapid loss in elevation is driven by the settling, compaction, and shrinking of the OM as it dries (i.e., primary consolidation), while microbial oxidation (conversion of the OM to CO_2) dominates during longer-term elevation loss (Deverel and Leighton, 2010; Drexler et al., 2009; Hooijer et al., 2012; Stephens and Speir, 1970; Wösten et al., 1997). Additionally, increased sunlight exposure during WLDD may have also played a role in altering OM properties and promoting decomposition, particularly of fresh litter material (Cory and Kling, 2018; Hunting et al., 2019).

The intact core experiment allowed for an investigation of the cause of the floc thickness reduction by providing data on initial and ending bulk density, initial and ending volume, and the position of the water table. Using the formula presented in Hooijer et al. (2012), an estimated 95–96 % of the loss in floc thickness was a result of primary consolidation, mirroring other studies that suggest physical processes drive elevation loss in drained organic soils immediately following drainage (e.g., Aich et al., 2013; Couwenberg et al., 2010; Franzén, 2006; Hooijer et al., 2012; Kool et al., 2006). For example, drained peatlands in SE Asia lost 60–100 cm of soil elevation in the first ~1–2 years after drainage due to dewatering and compaction, followed by a slowing of the rate to ~4–5 cm y^{-1} for the next few decades, which was driven primarily by oxidation (Deverel and Leighton, 2010; Drexler et al., 2009; Hooijer et al., 2012; Wösten et al., 1997).

4.2. Long dry-down without soil amendments/mixing maximized oxidation rate

Although oxidation was only responsible for 4–5 % of the reduction in floc thickness (according to the laboratory experiment) cumulative CO_2 flux was still substantial and positively related to the total number of days dry (Fig. 5c). Others have found drying-rewetting cycles can accelerate soil organic C mineralization and CO_2 emissions by creating a pulse of greater substrate availability (Borken and Matzner, 2009; Gao et al., 2016), but no clear effect of drying-rewetting cycles on CO_2 flux was observed in the Multiple Short treatment of this study. This could be because the already high availability of labile C and low C:N of this soil created an environment that was not substrate limited (Morillas et al., 2015) or that the intensity of drying was not extreme enough to have a significant effect on aggregate stability and/or microbial biomass (Borken and Matzner, 2009; Zhu and Cheng, 2013).

What did impact C mineralization rate, beyond just the total number of days of dry-down, was the addition of the clay amendment (i.e., Single Long Amended treatment), designed to replicate the 'hybrid de-mucking' rejuvenation method sometimes employed in the OEW. Indeed, the addition of 20 g of site clay to the top 10 cm of the Single Long Amended treatment increased the average mass of soil C in the MAOM fraction by 60 % and decreased the average rate of CO_2 flux by 36 %, relative to the Single Long (unamended) treatment. This suggests the clay addition (simulating the mixing of OM with the underlying mineral soil) can slow soil oxidation and the formation of MAOM was a contributing mechanism. Interestingly, the clay addition also stimulated the formation of macroaggregates (>2 mm) in the Single Long Amended treatment, with 5-times more C in this size fraction in the amended treatment, relative to the unamended. Although macroaggregates provide only minimal physical protection to OM compared to MAOM, it still may be more stable than loose floc, or free particulate organic matter (Six et al., 2002; von Lütow et al., 2007). Based on these findings, hybrid de-mucking, mixing the remaining OM with the underlying mineral soil, may slow the rate of soil oxidation and therefore be undesirable in the context of this study where rapid reductions in OM is the goal.

Despite the observed differences in CO_2 flux among the different

WLDD treatments tested in the laboratory experiment, none stood out as more effective in reducing the thickness of the floc layer than another (Fig. 6a). Like the field study design, all intact cores were re-flooded to the height of the control treatment for multiple days before being measured, deconstructed, and analyzed for biogeochemical properties. Even after re-flooding, all treatment cores averaged a 35–41 % reduction in floc thickness relative to before the experiment; meanwhile the floc thickness in the continually flooded Control remained unchanged. Bulk density did increase across all cores, which may be related to the artificial laboratory conditions and the death/exclusion of porous live plant roots (DeLaune et al., 1994). Also, the notable increase in bulk density and decrease in OM content of the Single Long Amended treatment was considered a consequence of dilution with the clay addition. Changes in soil elevation were not significant in the laboratory experiment.

4.3. Water level draw-down caused a temporary release of N and P

As a municipal CTW, the removal, transformation, and burial of N and P are of paramount interest at the study site; in fact, maximizing N and P removal efficiency is the goal behind the effort to reduce OM accumulation through improved sheet flow and longer residence times (Wang et al., 2006). Therefore, for WLDD to be an effective management tool, potential impacts to N and P removal rates need to be quantified both during and following the WLDD.

Whilst the floc layer dominated as the source of flow-obstruction in the CTWs, so too did it dominate as the primary nutrient reservoir. Wetland floc has been described by others as an “active interface” between the water column and underlying soil (Zamorano et al., 2018) and is known as a hotspot for bioavailable OM and the cycling and storage of C, nutrients, and metals (Neto et al., 2006; Noe et al., 2002; Zamorano et al., 2018). This was evident at the OEW field experiment where baseline analysis revealed the floc had exceedingly greater OM, extractable NO_x , NH_4^+ , and SRP per gram than the soil (Table 1). Drying of the floc layer was expected to enhance physical consolidation, while also accelerating decomposition as greater O_2 availability allowed for increased aerobic soil respiration (Moore and Dalva, 1993; Reddy and DeLaune, 2008; Smith et al., 2018). Previous wetland studies documented a 53 to 310 % increase in soil respiration rates when the water table was below the soil surface, as compared to when flooded, depending on the texture of the soil (Chambers et al., 2013). During OM mineralization, organic N and P are also released as inorganic compounds, which can subsequently be transformed, assimilated, or accumulate in the soil and water, depending on the conditions (Reddy and DeLaune, 2008).

The two dry events in the field experiment, and the inter-mixed reflooding due to precipitation, created a varied hydroperiod in the treatment cell. Drying and wetting cycles in soils are well known to work synergistically to promote coupled nitrification and denitrification, respectively, by shifting the oxidation-reduction status of the soil (Groffman and Tiedje, 1988). In this study, nitrification increased significantly in both the floc and soil of the treatment cell when it experienced the two dry events, as exemplified by almost 5 times more extractable NO_x in the floc layer when dry, and 21 times more NO_x in the soil layer when dry, relative to when the treatment cell was flooded. Dry event #1 also stimulated N mineralization, as demonstrated by extractable NH_4^+ concentration more than doubling in the floc. Previous wetlands studies have also observed greater NH_4^+ availability following an increase in oxygen as aerobic microbes are more efficient at mineralization (Steinmuller et al., 2019). Interestingly, the second dry event had the opposing effect and significantly reduced extractable NH_4^+ relative to both the control cell and initial concentrations, possibly an indicator of N assimilation by plants and microbes.

Although P is not a redox-active element, it is still affected by changes in redox potential due to WLDD through interactions with other elements (e.g., iron), the accompanying changes in pH, and shifts in the stoichiometric relationship with C and N (Reddy and DeLaune, 2008).

The dynamics of P cycling and storage were particularly important in this study because prior observations of reduced P removal efficiency within the OEW were the catalyst for beginning management activities to address OM accumulation, which was considered a causal factor in reduced P retention (Wang et al., 2006). In the field experiment, extractable SRP was unaltered by dry event #1, but increased roughly 2-fold in the floc and soil during dry event #2. The fact that the increase in SRP was asynchronous with the increase in NH_4^+ (i.e., NH_4^+ increased only during dry event #1, whereas SRP increased only in dry event #2; Table 1) suggests a decoupling of the N and P cycle in response to WLDD. Specifically, N cycling appeared driven by increased oxygen availability supporting accelerated nitrification and mineralization (indicated by elevated NO_3^- and NH_4^+ during dry events), while the delayed response of P may be more strongly linked to a decrease in pH and release of metal-bound P, rather than OM mineralization. Importantly, all extractable N and P concentrations in the floc and soil returned to values comparable to that of the control cell during the post-WLDD.

4.4. Water level draw-down is an effective management tool if used regularly

Assuming a linear accumulation rate, field data indicates floc thickness increases 1.8 to 2.7 cm annually in the OEW. Therefore, the observed 16.4 cm reduction in floc thickness achieved with two successive annual dry downs (20–22 days each in duration) represented a loss of roughly 7.5 years' worth of accumulated floc. If temporary WLDD were adopted as part of a routine hydrologic management scheme at the OEW (e.g., once every 3–5 years in each cell), our data suggests it would significantly reduce, or even terminate the need for costly mechanical cell renovation projects. Of note, a full dry-down event was difficult to achieve in this humid subtropical climate without the excavation of a temporary drainage canal and the short-term use of diesel-powered pump to remove excess water. However, with these additional tools two full ~3 week dry-downs were achieved in successive years, both during the region's dry season (mid-Oct-May). Targeting the dry season for WLDD events is most feasible and could provide additional benefits because the OEW will compete for water with residential reclaimed water demand as the population continues to grow. Demand for reclaimed water is heightened during the dry season, reducing the available supply of inflow water.

No permanent change in N removal efficiency was noted because of WLDD, but temporary enhancement of coupled nitrification-denitrification is inferred from reductions in floc DIN in both the field and laboratory studies when dry events occurred. For P, a short-term (~36 d) increase in the release of SRP in the surface water was documented at the outflow of the field treatment cell. The same effect was observed as a result of prescribed burning at the OEW, which was similarly tested as a management technique to remove excess accumulated OM. In the fire study, outflow SRP increased for approximately 23 d before returning the baseline conditions (White et al., 2008). However, burning had no documented reduction on floc thickness or soil elevation, but rather reduced aboveground biomass by ~68 % (White et al., 2008). Together, both studies demonstrate that management activities employed to accelerate OM mineralization need to plan for a short-term release of P. To prevent this P pulse from causing non-compliance with outflow P concentration limits, surface water should be held in the cell for approximately 4–5 weeks, allowing time for P re-assimilation, before opening the outflow discharge of the treated cell.

Although the primary function of the OEW is to reduce total N and P concentrations in the discharge water from the regional water reclamation facility, it has also developed into a critical wildlife habitat, popular park, and tourist attraction. Compared to a full renovation effort that scrapes a wetland treatment cell down to the native underlying soil and re-starts secondary succession with some manual plantings and natural recruitment, WLDD is significantly less invasive, less costly, and preserves much of the habitat during the management activity. The key

to successful implementation of a WLDD management regime to reduce OM accumulation is anticipated to be 1) acting before nutrient removal efficiency begins to decline, and 2) implementing it on a regular and consistent timescale.

5. Conclusions

Allowing for increased oxygen availability in water-logged soils is a well-established mechanism for promoting OM oxidation and removal, but quantifying the impact it will have on floc thickness and soil elevation, as well as N and P dynamics in a CTW, must be evaluated prior to implementation given the potential impacts to habitat and mandated nutrient removal. Returning to the study's initial hypotheses, our data supported the idea that WLDD could significantly decrease floc thickness relative to the control and initial measurements under field conditions. When dry, floc thickness averaged ~83 % less than both the control cell and initial measurements. Likewise, when dry, soil elevation average 5.2 cm less than the initial, which was also a greater loss than the control cell elevation (which remained unchanged). As hypothesized, the observed decreases in the treatment cell did persist even 2 months after reflooding, with a final average floc thickness reduction of 60 % and elevation loss of 2.7 cm. The WLDD did generally cause a temporary increase in extractable NO_x , NH_4^+ , SRP, and DOC in the floc, and to a lesser degree in the soil, as expected due to greater mineralization when oxygen availability increases. However, as hypothesized, all extractable nutrient concentrations return to baseline conditions by the post-WLDD sampling. In terms of the impact to N and P in surface water, our hypothesis of a short-term spike following reflooding was only supported for SRP, which was elevated relative to the control cell for ~36 days before returning to similar concentrations. Neither surface water NO_x nor NH_4^+ outflow concentrations were altered by the WLDD, which differed for our hypothesis that N removal efficiency may temporarily decline.

For the laboratory intact soil core experiment, multiple cycles of WLDD did not accelerate mineralization through CO_2 loss, but rather CO_2 loss was directly related to the total number of days dry (regardless of wet-dry cycling). Floc resuspension was observed following reflooding of the intact soil cores, but the final thickness averaged ~36–41 % less than the control and initial thickness in all treatments receiving any WLDD; this reduction did not differ significantly by WLDD treatment. Finally, mixing the floc and soil OM with a small amount of clay amendment during the WLDD did result in greater soil macroaggregate and MAOM formation (indicative of physical and chemical protection of the OM), which was correlated with a lower (~36 % less) cumulative CO_2 loss, making this technique less desirable from the standpoint of maximizing OM removal.

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CRediT authorship contribution statement

Paul Boudreau: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mark Sees:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Anthony J. Mirabito:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Lisa G. Chambers:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no competing or conflicting interests to declare.

Data availability

Data will be made available on request.

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